American Journal of Hematology 56:29–31 (1997)

Activated Protein C Correlates Inversely With Thrombin Levels in Resting Healthy Individuals

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To study whether the circulating anticoagulant, activated protein C (APC), could be a regulator of thrombin activity in basal physiological conditions, fibrinopeptide A and activated protein C levels were determined in samples from 40 healthy individuals. There was a significant inverse correlation between the fibrinopeptide A and APC levels (Spearman rank correlation R = −0.487; *P* = 0.0023). Because of well-known mechanisms by which decreasing APC levels could cause increased thrombin formation, we suggest that APC may downregulate thrombin activity in subjects with normal protein C levels. Regulation of thrombin formation in health is likely significant for maintaining vascular patency but its molecular mechanisms are poorly understood. The current data suggest that a single physiological anticoagulant, namely APC, may be a significant regulator of procoagulant thrombin activity. Am. J. Hematol. 56:29–31, 1997.

Key words: protein C; thrombin; fibrinopeptide A; anticoagulants; venous thrombosis

INTRODUCTION

Congenital defects of the protein C pathway, including protein C and protein S deficiencies, as well as resistance to activated protein C (APC) caused by the R506Q mutation in coagulation factor V, are risk factors for venous thrombosis [1–3]. APC, but not its inactive precursor protein C, proteolytically inactivates coagulation factor Va in vitro, which decreases prothrombin activation by factor Xa [4]. Individuals with genetic defects of the protein C pathway have increased levels of prothrombin fragment 1 + 2, reflecting increased thrombin formation and their hypercoagulable state [5–7]. However, it remains unknown whether APC, which normally circulates at about 40 pM concentration [8], regulates thrombin formation under basal physiological conditions. In fact, it is poorly understood which activation mechanisms of coagulation are responsible for the basal thrombin activity and, likewise, which anticoagulant mechanisms downregulate thrombin formation in health.

To evaluate whether increasing levels of APC would downregulate procoagulant thrombin activity, direct measurement of APC instead of zymogen protein C is required because thrombin itself binds to thrombomodulin and activates protein C to APC [9]. To quantitate the procoagulant thrombin activity, we chose to measure fibrinopeptide A (FPA) [10] since FPA is released by thrombin cleavage of fibrinogen. It should be noted that coagulation markers such as prothrombin fragment F 1 + 2 or thrombin-antithrombin complexes reflect total thrombin formation [11] and only part of the thrombin formed will cleave fibrinogen.

MATERIALS AND METHODS

Plasma samples from 40 apparently healthy individuals [19 men and 21 women, mean age 33.7 (range 22 to 59 years)] were studied. Two 2.7-ml venous blood samples were collected after an overnight fast into two

Contract grant sponsor: Cigarette and Tobacco Surtax Fund of the State of California through the Tobacco-Related Disease Research Program of the University of California; contract grant numbers: 1RT304 and 2RT342; contract grant sponsor: National Institute of Health; contract grant number: HL 52246; contract grant sponsors: Stein Endowmen Fund; Sigrid Jusélius Foundation; Paulo Foundation; Finnish Cultural Foundation.

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Received for publication 16 April 1997; Accepted 7 May 1997.

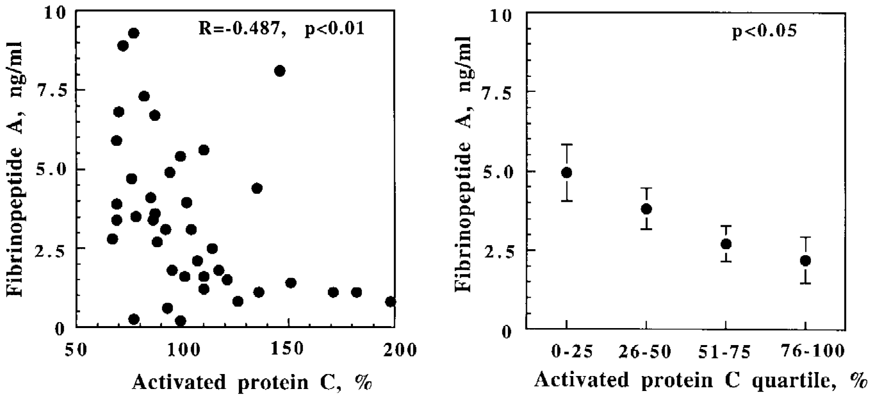

Fig. 1. Fibrinopeptide A levels as a function of activated protein C levels in 40 healthy adults. Left: Activated protein C as percentage of normal plasma pool. The Spearman rank correlation coefficient with associated *P* value is presented. Right: The 40 subjects were grouped into quartiles (10 subjects in each) according to the increasing activated protein C level. Mean ± standard error of mean are given for each quartile. The *P* value was calculated with the Kruskal-Wallis test.

prechilled 3-ml syringes, each containing 0.3 ml of anticoagulant. The anticoagulant in the first syringe contained a mixture of 0.3 M benzamidine and 0.2 M EDTA. The other syringe contained 0.11 M trisodium citrate. Plasma was separated by centrifugation at 12,000g for 2 min at 4°C, aliquots were frozen in liquid nitrogen and stored at −80°C. Before FPA measurement from the benzamidine-EDTA anticoagulated samples, fibrinogen was removed by centrifuging sample aliquots in 0.5 ml spin vials fitted with 30,000 molecular weight cut off membranes (Gelman Sciences, Ann Arbor, MI) that retain macromolecular species, including fibrinogen, from plasma. Fibrinogen-depleted samples were then assayed using Asserachrom FPA™ kit (American Diagnostics, Parsippany, NJ). Clottable fibrinogen was measured as described [12]. The circulating levels of APC were determined using an enzyme capture assay as described previously [8]. Briefly, antiprotein C monoclonal antibodies were immobilized in microplates. Plasma samples containing benzamidine, a reversible inhibitor of APC, were incubated in the wells for capture of APC antigen. Unbound sample constituents and the benzamidine were removed by extensive washing. Finally, the amidolytic activity of the captured APC was measured using chromogenic substrate S-2366 (Chromogenix AB, Mölndhal, Sweden). The level of activatable protein C was measured as described [8]. The Spearman rank correlation coefficient and the Kruskal-Wallis test were used for statistical evaluation; $P < 0.05$ was regarded as significant.

RESULTS

All subjects had normal protein C levels (99 ± 3.4%, mean ± standard error of mean) suggesting no congenital protein C deficiency in the study group. There was a significant inverse non-linear correlation between the APC and FPA levels (Fig. 1, left). When the study group was divided into quartiles according to the APC levels, the significant decrease in the FPA levels as a function of increasing APC level became readily demonstrated (Fig. 1, right). The correlation between FPA and fibrinogen levels was non-significant ($R = 0.283$, $P > 0.05$), reflecting the significance of thrombin activity rather than fibrinogen availability in fibrin formation.

DISCUSSION

In accordance with our hypothesis, we found a significant inverse correlation between circulating APC and FPA levels. Although we cannot demonstrate whether this correlation reflects a causal relationship or whether it reflects two unrelated effects, each due to a third unknown independent variable, we favor a causal association as the most likely interpretation because of the well-known inverse relationship between increasing APC and decreasing rates of thrombin generation in purified systems. Accordingly, we suggest that even minor physiological or pharmacologically induced variations in circulating APC levels may significantly contribute to the basal levels of procoagulant thrombin in vivo. This could have significant implications for research, prevention, and therapy of venous or arterial thrombosis in the population whether or not individuals are free of diagnosable thrombophilic coagulopathies. To summarize, the current data suggest that of the multiple mechanisms of physiological anticoagulation, a single factor, namely APC, may be quantitatively important enough to regulate procoagulant thrombin activity [13] even in a study of normal nonsmokers with limited sample size like this one.

ACKNOWLEDGMENTS

This work was supported by grants from the Cigarette and Tobacco Surtax Fund of the State of California through the Tobacco-Related Disease Research Program of the University of California (1RT304 and 2RT342), the National Institute of Health (HL 52246), the Stein Endowment Fund, the Sigrid Jusélius Foundation, the Paulo Foundation, and the Finnish Cultural Foundation.

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